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STUDIES ON THE VIRUS OF TYPHUS.

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DURATION OF THE INFECTIVITY OF THE BLOOD.

The human inoculations by Moczutkowski (1900) and Otero (1908) strongly suggested, and those of Yersin and Vassal (1908) made it highly probable,[1] that the typhus-fever virus resides in the blood. Satisfactory proof of this, however, was not adduced until February, 1910, when Anderson and Goldberger showed that the fever induced in the monkey by an inoculation with human typhus blood, besides being transmissible by passage, conferred a definite immunity to a second inoculation.[2]

Since that time the experimental work of Anderson and Goldberger, of Ricketts and Wilder, of Gaviño and Girard, but more particularly that of Nicolle and Conseil, has developed a solid basis for the conclusion that the virus of typhus may be (or is) present in the blood, at least throughout the febrile period.

Nicolle and Conseil (August, 1910) were the first to try to determine whether the virus is present in the circulating blood before the onset of fever and after its defervescence, and from their experiments they concluded (January, 1911) that typhus blood is virulent, not only throughout the febrile period, but also before the onset of fever and at the beginning of convalescence (second day after defervescence).

In our recent work we repeated Nicolle and Conseil's experiments and have obtained results that cast grave doubt on the validity of Nicolle and Conseil's interpretations.

Before presenting our own work it will be desirable to examine Nicolle and Conseil's experiments somewhat in detail.

In order to determine whether typhus (monkey) blood is still infective after defervescence, Nicolle and Conseil inoculated a bonnet monkey (No. 31) with some blood from a chimpanzee (No. 3) obtained 36 to 48 hours after the return to normal of its temperature. After an incubation of 8 days the temperature of the bonnet monkey rose abruptly, remained up about 24 hours, then dropped, without

[1] There is some doubt whether Yersin and Vassal were really working with typhus.

[2] Nicolle's proof of the successful inoculation of his chimpanzee was the occurrence of a mild fever 24 days after inoculation and an eruption. Although the injection of 1 c. c. of the chimpanzee's blood was followed, in a bonnet monkey, by fever "with a most characteristic eruption," this was hardly convincing proof that the fever was typhus. It may be remarked in this connection that in his later work Nicolle has ceased to note the occurrence of an eruption.

subsequent hypothermia, but with slight emaciation. This is interpreted by Nicolle and Conseil as a typhus reaction, although when later subjected to an immunity test bonnet monkey 31 presented a well-marked reaction, showing that the fever of some 24 hours' duration, which Nicolle and Conseil interpret as a typhus reaction, had conferred no immunity.

With a view of testing the infectivity of the blood during the incubation of typhus, Nicolle and Conseil inoculated a bonnet monkey (No. 37) with blood from a chimpanzee (No. 4) obtained 3 days before the beginning of the fever. The result of this inoculation appeared to be absolutely negative, but when subjected to an immunity test 33 days later this animal (bonnet monkey 37) did not react. Whereupon Nicolle and Conseil conclude that the first inoculation, although followed by no symptoms, had conferred on their bonnet 37 a solid immunity.

It is evident that Nicolle and Conseil's conclusions that the blood is infective after defervescence and during the prefebrile period depend on the validity of their interpretations of the two experiments just cited.

The validity of their interpretation of the first of the above experiments is clearly involved in the question of what constitutes a typhus reaction. We defer a full discussion of this question to a later paper. At this time we wish simply to state that we are convinced that in the present state of our knowledge it is not permissible to interpret as a typhus reaction in the monkey a fever that confers no immunity.

In the second of the two experiments cited Nicolle and Conseil base their interpretation on the outcome of the immunity test; as this was negative they conclude that the animal, though it had presented no symptoms, was vaccinated by the injection with the blood obtained from chimpanzee No. 4.

It is obvious that in this case Nicolle and Conseil disregard the possibility of a natural immunity in the monkey. This possibility is not as remote as the early workers, including ourselves, believed. We have met with it several times in our recent work. Instances will be cited in other portions of this paper, although we shall defer a full discussion of this important matter to a later communication. For our present purpose it will be enough to say that in several instances in our recent experience monkeys have resisted one or two inoculations with virulent blood or blood serum and yet have reacted sharply and characteristically to the second or the third.

From the foregoing discussion it is clear that Nicolle and Conseil's conclusions are not justified by the results of their experiments when these are strictly interpreted.

Our own experiments follow:

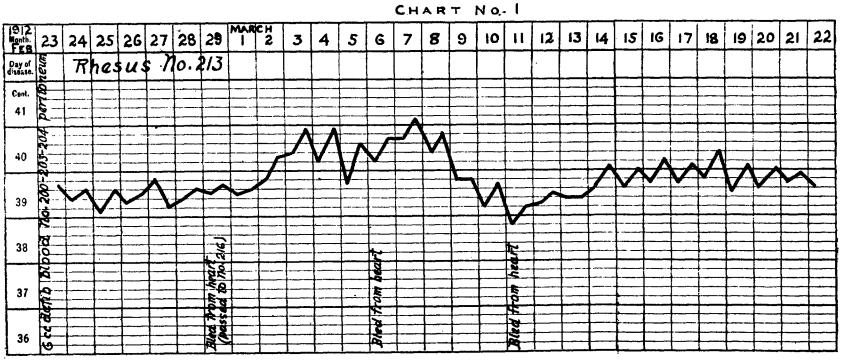
Experiment No. 1.

At 12 m., February 29, about 32 hours before the beginning of a well-marked typhus reaction, we aspirated blood from the heart of rhesus No. 213 (chart 1) and after defibrination injected 8 c. c. of it diluted with 4 c. c. of saline solution into the peritoneal cavity of rhesus No. 216.

Result: During a period of observation of 30 days rhesus No. 216 failed to give any evidence of a reaction. At the end of this period he was subjected to an immunity test consisting of an intraperitoneal injection of 3 c. c. of defibrinated blood, obtained from monkeys Nos. 232 and 233, diluted with 2 c. c. of saline solution. To this test rhesus No. 216 reacted promptly and characteristically.

It appears, therefore, that monkey No. 216 was neither infected nor vaccinated by the inoculation with the blood of No. 213. This result would seem to justify the interpretation that the blood obtained from rhesus No. 213 about 32 hours before the beginning of fever was

CHART No. 1



Temperature curve of rhesus No. 213.

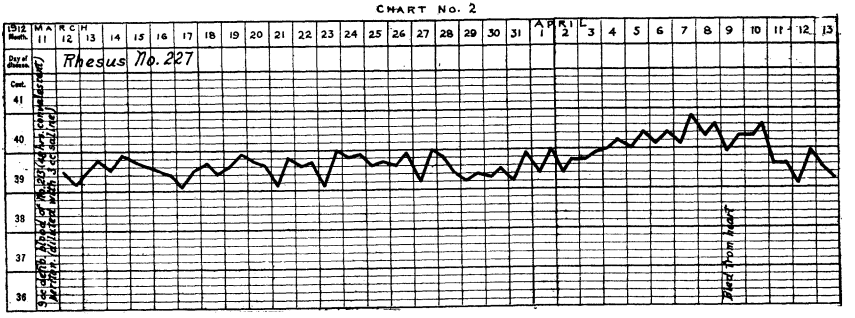
not virulent. This interpretation, though probably correct, is not permissible, in view of our experience that the normal monkey may resist one or two or more inoculations with virulent blood.

Experiment No. 2.

At 3.50 p. m., March 11, 1912—that is, at least 24 to 32 hours after the temperature of rhesus No. 213 reached normal—we again drew blood from this animal's heart and after defibrination, 9 c. c. of it diluted with 3 c. c. of saline solution was injected intraperitoneally into rhesus No. 227.

Result.—After an incubation period of about 24 days this monkey developed a mild fever of about 7 days' duration, which is sufficiently definite to justify us in considering it a typhus reaction (chart 2).

CHART No. 2



Temperature curve of rhesus No. 227. Mild febrile reaction following inoculation with blood obtained in beginning of convalescence.

The mild reaction obtained in rhesus No. 227 following inoculation with blood obtained at least 24 to 32 hours after the temperature of rhesus No. 213 reached normal justifies the interpretation that this blood was infective.

CONCLUSIONS.—From the foregoing discussion and the results of our experiments the following conclusions seem to us permissible:

1. The blood of the monkey infected with typhus may be virulent in the prefebrile stage, but no satisfactory evidence of that fact has as yet been adduced.

2. The blood of the monkey may still be virulent 24 to 32 hours after the return of the temperature to normal.

THE SEAT OF THE VIRUS OF TYPHUS.

The demonstration of the presence of the typhus virus in the circulating blood naturally leads one to inquire as to the element of the blood in which it resides. Nicolle and Jaeggy (April, 1910) were the first to consider this problem. In their hematologic study of typhus they describe a peculiar necrosis of the polynuclears which they consider significant as regards the seat of the unknown infectious agent, an agent which they therefore think is probably an intraleucocytic parasite.

Somewhat later Nicolle, Conor, and Conseil (October, 1910) point out certain apparent differences between the infectivity of typhus blood serum obtained after centrifugation of defibrinated blood and that obtained after clotting. These differences appear to them to support the intracellular hypothesis first suggested by Nicolle and Jaeggy.

In a later paper (January, 1911) Nicolle, Conor, and Conseil write that if the hypothesis of the intracellular character of the virus be accepted—

> the discordant results of inoculation with serum obtained by centrifugation of defibrinated blood and that obtained after coagulation are easily explained. The former is always virulent because no matter how rapid and perfect the centrifugation may have been, it is bound to contain cellular débris and organisms liberated by the destruction of cells in the act of defibrination. The serum obtained after coagulation is, theoretically, deprived of all virulence for the monkey because it is freed of most of the liberated organisms and almost completely of the suspended cells by the formation and retraction of the clot.

They add, however, that—

> the absence of virulence of the serum obtained after coagulation may not be absolute. When the serum does not separate well from the clot and when for that reason it contains a certain number of cells or when the amount inoculated is large the infection of the monkey may be realized.

In still a later paper (September, 1911) Nicolle, Conor, and Conseil report experiments especially designed to test their hypothesis. They consider that the results of these experiments support the theory of intraleucocytic localization.

On account of the great importance of the question involved it seems desirable to examine these experiments in detail. They are as follows:

A. COMPARATIVE VIRULENCE OF THE VARIOUS ELEMENTS OF THE BLOOD SEPARATED BY CENTRIFUGATION.

Experiment 1.—The blood used was drawn by venepuncture of patient 120 in the seventh day of a severe typhus, to which was added some sodium citrate, centrifugated,[1] etc. The manipulation of separation and lavage of the different elements of the blood took three-quarters of an hour.

[1] Just as we were about to go to press Nicolle, Conor, and Conseil's paper (Apr. 25, 1912) came to our hands, from which we cite the following additional details of this experiment: "After centrifugation (lasting 12 minutes) we at once removed several cubic centimeters of plasma from the upper portion of the tubes. The leucocyte layer was carefully removed and put in saline solution; several cubic centimeters of red corpuscles were then removed from the deepest portion of the tubes and placed in some of the same solution. These manipulations required 22 minutes. After that the white and the red globules suspended in saline solution were submitted to a second centrifugation of 10 minutes duration. This done, it required 6 minutes more to separate the cells from the liquid in which they were washed."

Seven bonnet monkeys were inoculated intraperitoneally as follows: A fresh control and two immunes each with 5 c. c. of the fresh blood; monkey A with the same dose of citrated blood; monkey B with the same dose of citrated plasma; monkey C with 2.5 c. c. of red corpuscles; monkey D with 1 c. mm. of the leucocyte layer.

The results were as follows: Fresh control (fresh blood), incubation 16 days, typhus of median severity of 12 days' duration (the temperature was over 40° during 6 days); immunes (fresh blood), negative. A (citrated blood), incubation 12 days, typhus of median severity of 9 days' duration (the temperature was over 40° during 9 days); B (citrated plasma), incubation 8 days, duration 9 days (6 days above 40°); C (red corpuscles), incubation 13 days, typhus abortive of 5 days (3 days at 40°, of which only one above); D (white corpuscles), incubation 6 days, typhus grave of 9 days (9 days above 40°).

Experiment No. 2.—Performed under the same conditions as No. 1 with the blood of patient 69 in the thirteenth day of a severe attack. Separation of the various elements of the blood took 30 minutes and was more perfect than in the first experiment. Three bonnet monkeys were inoculated intraperitoneally as follows: Monkey E with 5 c. c. of citrated plasma, F with the same dose of red corpuscles, G with about 1 c. mm. of leucocytes.

The results were as follows: E (citrated plasma), incubation 13 days, mild typhus of 7 days' duration (3 days above 40°); F (red corpuscles), negative; G (white corpuscles), incubation 7 days, severe typhus of 11 days (temperature above 40° during 9 days). Tested 67 days after the first inoculation by the intraperitoneal injection of 5 c. c. of blood from a monkey in the fifth day of an experimental attack; E and G have not reacted, F developed a severe typhus of 8 days after an incubation of 11 days.

B. NONVIRULENCE OF BLOOD SERUM FREED OF ITS CELLULAR ELEMENTS BY CENTRIFUGATION.

Serum obtained after clotting is, theoretically at least, free of cells. These remain entangled in the clot as it retracts; prolonged centrifugation frees it of such formed elements as it may contain. It is easy therefore to obtain a serum free of all white corpuscles. If our hypothesis as to the localization of the typhus virus in these cells is correct, such a liquid will be inoffensive. The experiment performed on man shows that it is so.

One of us received intravenously 1 c. c. of blood serum from a monkey in the third day of typhus seven hours after the blood was drawn; result negative. A previous experiment had shown us that the blood serum has no microbicidal action in vitro on the typhus virus.

C. NONVIRULENCE OF THE CEREBROSPINAL FLUID.

This liquid, destitute of all cellular elements, possesses no virulence.

"A bonnet monkey inoculated intraperitoneally with 15 c. c. of cerebrospinal fluid of patient 28 in the ninth day of a severe attack has presented no symptoms; tested 14 days later by an intraperitoneal inoculation of 5 c. c. of blood of a monkey in the fifth day, this monkey developed a fever of 7 days duration after an incubation period of 12 days."

From these experiments Nicolle, Conor, and Conseil draw the following conclusions:

1. Of the various elements of the blood separated by centrifugation the leucocytes are the most virulent; a minimal dose of these cells promptly determines in the monkey a grave infection.
2. The plasma, less active, seems to owe its virulence only to the leucocytes or leucocytic débris, of which it is difficult to free the plasma completely; the washed red cells are not virulent.
3. Centrifugated blood serum is inoffensive for man and a humor destitute of cells; the cerebrospinal fluid is equally inactive.

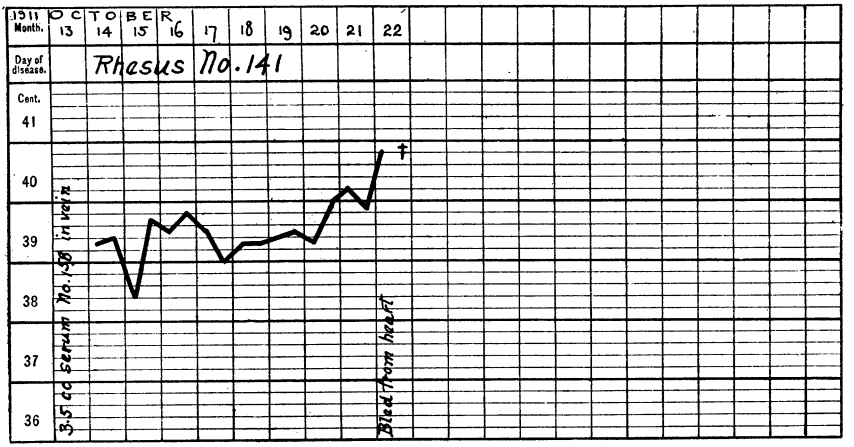
Before discussing the foregoing experiments and conclusions we desire to detail some pertinent experiments of our own.

Experiment No. 3.

On October 13, 1911, some defibrinated blood from rhesus No. 158, then in the first day of a typhus reaction, was centrifugated for about 30 minutes, after which the clear supernatant serum was pipetted off. With some of this serum two rhesus monkeys were inoculated. No. 141 received 3.5 c. c. intravenously; No. 142 received 0.5 c. c. subcutaneously and 4 c. c. intraperitoneally.

Result: Rhesus No. 141 developed a sharp reaction 7 days after the inoculation (chart 3); but during a period of observation of 29 days rhesus No. 142 gave no evidence of a reaction. At the end of this period No. 142 was subjected to an immunity test consisting of an intravenous injection of 2.5 c. c. of defibrinated blood of rhesus No. 163, to which he responded promptly and sharply. (Chart 4.)

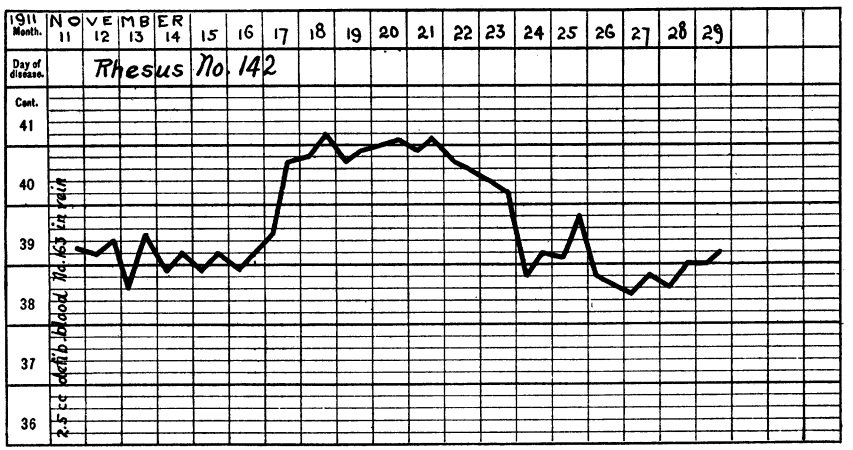
CHART No. 3



Temperature curve of rhesus No. 141, showing first three days of typhus reaction following inoculation with blood serum.

Although one of the two animals inoculated (with the larger dose) did not respond and was not vaccinated, it is clear that the blood serum of rhesus No. 158 was infective.

CHART No. 4



Temperature curve of rhesus No. 142, showing reaction to immunity test.

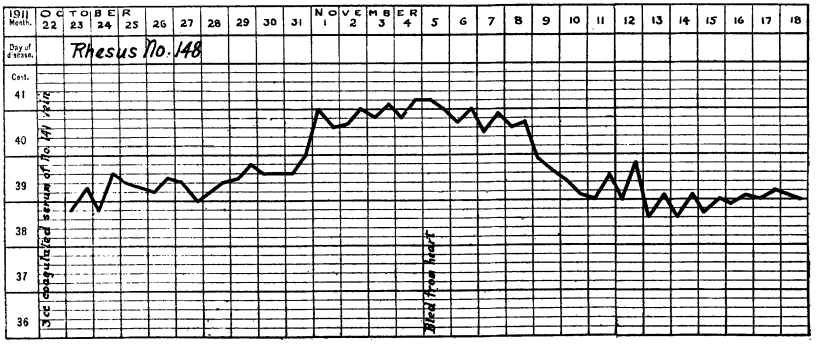
Experiment No. 4.

On October 22, 1911, blood was drawn from rhesus No. 141, then in the third day of a marked attack of experimental typhus (chart 3), and allowed to clot in a tube. After 3 hours, in order to separate the serum more thoroughly from the clot, the tube was centrifugated for about 15 minutes. After drawing off the clear straw-tinted serum, a portion of it was used for the inoculation of two monkeys. No. 147 was given

4.5 c. c. and No. 148, 3 c. c., both intravenously. The time that elapsed between drawing the blood and inoculating was about 3 hours and 20 minutes.

Result: During a period of observation of 29 days rhesus No. 147 gave no evidence of a reaction. Rhesus No. 148, however, after an incubation period of about nine days, developed a severe typhus, as shown by marked fever (chart 5) and successful passage to another monkey. It should be noted that, although rhesus No. 147 and rhesus No. 148 were of substantially the same size and vigor, yet rhesus No. 147—the one

CHART No. 5.

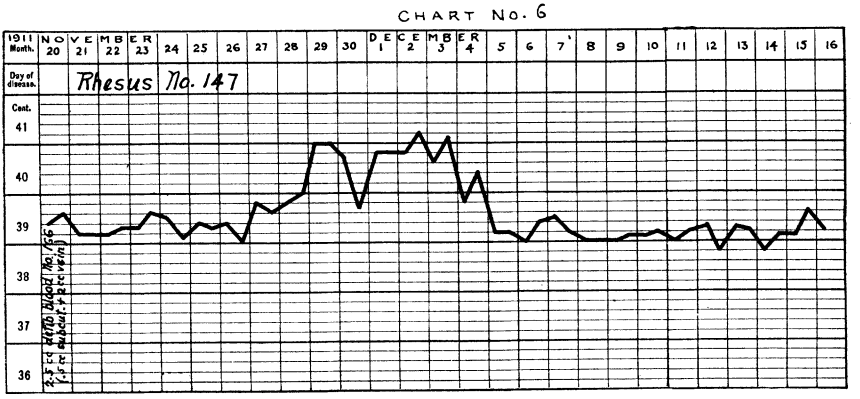


Temperature curve of rhesus No. 148, showing severe febrile reaction, following inoculation with blood serum.

that received the larger dose (larger by 50 per cent)—failed to react, and when subjected to an immunity test, consisting of a subcutaneous injection of about 0.5 c. c., and of an intravenous injection of 2 c. c., of defibrinated blood of rhesus No. 166, then in the third day of an experimental typhus, rhesus No. 147 reacted promptly and sharply (chart 6).

Although one of the two animals inoculated (with much the larger dose) did not become infected nor immunized, it is evident that the blood serum of rhesus No. 141, obtained after clotting and centrifugation, was infective.

CHART No. 6



Temperature curve of rhesus No. 147, showing reaction to immunity test.

Experiment No. 5.

On March 6, 1912, blood was aspirated from rhesus No. 210 and No. 213. The former was in the seventh and the latter in the fifth day of an experimental typhus. The blood was allowed to clot in tubes and, as in Experiment No. 4, after standing three hours it was centrifugated in order better to separate the serum from the clot. The serum was then pipetted off and mixed. Of this serum, 6 c. c. were intraperitoneally injected into rhesus No. 221.

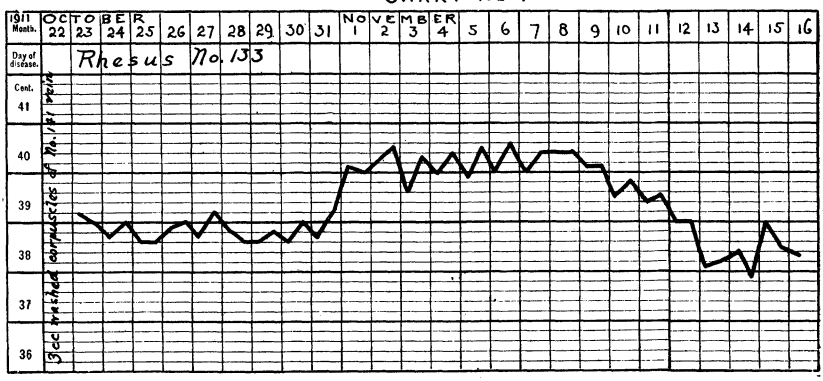
Result: During a period of observation of 24 days following this inoculation monkey No. 221 manifested no recognizable symptoms of a reaction. He was therefore reinoculated March 30 in order to test his immunity; for this purpose he was given an intraperitoneal injection of 3 c. c. of virulent defibrinated blood (rhesus Nos. 232 and 233). Twenty-one days later, April 20, not having in the meantime given any indications of a reaction, he was again given an intraperitoneal injection of 3 c. c. of virulent defibrinated blood (rhesus No. 225). So far this monkey has given no recognizable evidence of a reaction following this inoculation.

The results of the repeated immunity tests of rhesus No. 221 would seem to indicate that this animal was vaccinated by the first inoculation with 6 c. c. of serum; we think it more probable, however, that this is an instance of natural immunity in this monkey.

Experiment No. 6.

On October 27, 1911, some blood drawn from rhesus No. 141 (chart 3) was defribrinated and 3 c. c. of it tubed and centrifugated. After a centrifugation of about 15 minutes the serum was pipetted off and replaced by 5 c. c. of saline solution in which the corpuscles were thoroughly stirred up. The tube was then again put in the centrifuge for 15 minutes, after which it was taken out and the supernatant liquid pipetted

CHART No. 7



Temperature curve of rhesus No. 133, showing reaction following inoculation with washed corpuscles.

off and replaced by 5 c. c. of fresh saline solution. This maneuver was twice repeated. In other words, the corpuscles were washed three times in an excess of saline solution. After the third washing the corpuscles, in fresh saline solution, representing originally 3 c. c. of defibrinated blood, were injected intravenously into rhesus No. 133.

Result: After an incubation period of about 10 days this monkey developed a marked typhus reaction (chart No. 7).

Washing the blood corpuscles three times in an excess of saline solution had, therefore, not deprived them of the power to infect.

Experiment No. 7.

On February 12, 1912, we aspirated blood from three monkeys—Nos. 196, 198, and 322—that were in the fourth, fifth, and sixth days, respectively, of experimental typhus. The blood was defibrinated and mixed. The corpuscles of 12 c. c. of this mixed defibrinated blood were washed three times, as in experiment No. 6. After the third washing the supernatant liquid was drawn off and discarded; then the surface layer ("cream" or leucocyte layer) of corpuscles was pipetted off and injected intraperitoneally into rhesus No. 206. Of the corpuscles remaining, 3 c. c. were intraperitoneally injected into rhesus No. 207.

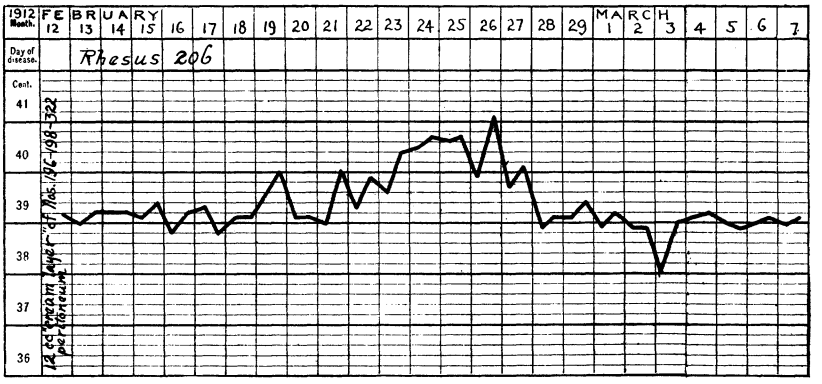
Result: Both monkeys developed well-marked reactions after an incubation period of seven days in No. 206 and eight days in No. 207. Although the reaction in neither was severe, that in No. 207 was perhaps the somewhat better marked (charts 8 and 9).

Both the "cream" or leucocyte layer and the red [1] corpuscles of washed blood were, therefore, infective.

DISCUSSION.—Nicolle, Conor, and Conseils's interpretations are evidently based on the assumption that the severity of the reaction is a direct index of the virulence of the infecting dose.

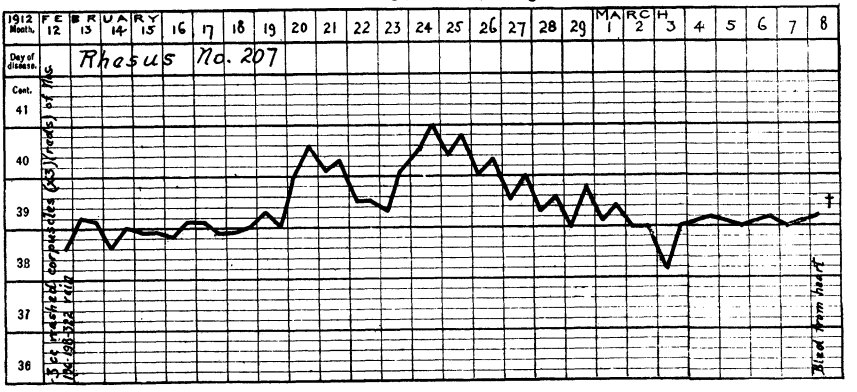
If this assumption were permissible it would be difficult to say from the character of the reaction noted by us in rhesus No. 206 and No.

CHART No. 8



Temperature curve of rhesus No. 206, showing reaction following an inoculation with leucocyte layer of washed corpuscles.

CHART No. 9



Temperature curve of rhesus No. 207, showing reaction following an inoculation with washed "red" corpuscles.

207 (charts 8 and 9) which was the more virulent, the "cream" layer given to No. 206 or the "reds" given to No. 207. As a matter of fact,

[1] Although centrifugation separates the white and the red corpuscles, this separation is never complete. A considerable number of leucocytes are always carried down by the reds from which they can not be completely removed, even by repeated washings and repeated removal of the "cream" layer. So that when we speak of the "reds" it must always be understood that a not inconsiderable number of whites are included. This is well illustrated by the following experiment, kindly made for us by our colleague, Dr. Leake: Some typhus monkey blood was citrated and then centrifugated for one hour. After this the serum and the cream layers were pipetted off and the remaining "reds" washed three times. After each washing the "cream" layer that formed was discarded. Leucocyte counts were made as follows: (1) Citrated blood before centrifugation, 7,000 per c. mm.; (2) plasma after centrifugation of 1 hour, less than 10 per c. mm.; (3) "cream" layer after centrifugation of 1 hour, 79,000 per c. mm.; (4) "reds" after centrifugation 1 hour and after removing the "cream," 14,000 per c. mm.; (5) "reds" after washing three times and each time discarding the "cream" layer, 9,000 per c. mm. After an hour's centrifugation, therefore, the plasma of citrated blood was not freed of leucocytes and the number of leucocytes among the "reds" was relatively increased.

there does not appear to be any necessary relation between dosage and severity of reaction

Ricketts and Wilder (Feb. 5, 1910) and Anderson and Goldberger (Feb. 18, 1910), almost simultaneously record observations to this effect over two years ago. We have an extreme and a very pertinent illustration of this in the result above detailed of our experiment No. 4 in which, of two animals of equal size and vigor inoculated with typhus blood serum, the monkey that received the smaller dose presented a severe reaction while the monkey that received the larger dose (by 50 per cent) failed altogether to react although a month later he reacted promptly and sharply to an immunity test with a relatively small amount of defibrinated blood. But waiving this objection and accepting their interpretation that "of the various elements of the blood separated by centrifugation the leucocytes are the most virulent," "the plasma less active," "and the washed corpuscles not virulent," ("not virulent" in the sense, it is presumed of "low virulence," for in one of their experiments under "A" the "reds" which necessarily entangled some leucocytes, were infective, as they were also in our experiment No. 7), it does not seem to us necessarily to follow that because the degree of virulence of the various elements of the blood thus separated appears to run parallel [1] with their (incorrectly) assumed richness in leucocytes that the virus is localized in the leucocytes or has any relation to the leucocytes other than that of possessing, possibly, a specific gravity approximating, or identical with, that of these cells.

In other words, having due regard for the mechanics of centrifugation, one might reasonably expect that the distribution in the different layers of centrifugated blood of an extracellular parasite of approximately the same specific gravity as that of the leucocytes would be similar to, or identical with, that of the latter. This is well illustrated by the following experiment. Some blood was drawn into sodium citrate solution from the heart of a guinea pig infected with anthrax. A measured amount of this was plated in agar. The remaining citrated blood was then centrifugated for one hour, after which measured amounts of the different layers, plasma, "cream," and "reds," uniform with that of the whole blood, were plated. After incubating 18 hours at 37° the plates were examined. The dilutions not having been sufficient to permit counting the colonies on some of the plates, estimates of their number were made and then certain relative values given to each, with the following result: "Whole blood," 100; "plasma," 1; "cream," 10,000; "reds," 10. Here we have an enormous excess of organisms in the leucocyte layer and a great reduction in the plasma and in the "reds." The reason for the high virulence of the leucocytic layer, the lessened activity of the plasma and the "nonvirulence" [2] of the washed red corpuscles is therefore obvious.

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[1] As a matter of fact, there is no evidence to show that it really does, for the leucocyte content of the various "elements" of the blood separated by centrifugation, is in the following order: "Cream," "reds," plasma (see note, Experiment No. 7).

[2] Nicolle, Conor, and Conseil say: "Des divers éléments du sang, séparés par centrifugation, les globules blancs sont en effet les plus virulents; une dose minime de ces cellules détermine chez le singe une infection rapide et grave; le plasma, moins actif, semble ne devoir sa virulence qu'aux leucocytes ou débris leucocytaires dont il est malaisé de le débarrasser complètement; les globules rouges lavés n'ont pas de virulence."

We presume that by "n'ont pas de virulence"—have no virulence—they mean "low virulence," for in one of their experiments under "A" the "reds" were infective. A literal interpretation of this phrase would make their argument in favor of an intraleucocytic localization absurd, for the "reds" necessarily entangle a not inconsiderable number of leucocytes as they are precipitated by the centrifuge.

If now we bear in mind that the severity of the reaction does not necessarily correspond to the degree of virulence (dosage), even though it may do so under certain circumstances, then we believe we have accurately reproduced in this experiment with anthrax blood the results obtained by Nicolle, Conor, and Conseil after centrifugation of citrated typhus blood.

In view of the foregoing it is easy to conceive that typhus blood serum may by sufficient centrifugation be made noninfective even to so (supposedly) sensitive a subject as man. On account of the variable susceptibility of the monkey, very little significance can be attached to the negative result of the single inoculation with cerebrospinal fluid. It does not prove that this fluid is not infective, although it is in harmony with the result of a human experiment made by Otero in 1908. But even if it did, to have the significance that Nicolle, Conor, and Conseil seem to attach to it, it would be necessary to show, first, that the cerebrospinal fluid of typhus is quite free of leucocytes, and second, that in infections due to an extracellular parasite this fluid is invariably virulent.

CONCLUSIONS.—From the foregoing discussion we believe it permissible to conclude: (1) That the evidence adduced by Nicolle, Conor, and Conseil does not especially favor their hypothesis of an intraleucocytic localization of the virus of typhus. On the contrary, the infectivity of centrifugated blood serum, obtained after clotting, with its low leucocyte content would be in favor of a parasite free in the circulating plasma of the blood; (2) that the blood serum of virulent typhus blood is constantly infective, whether obtained from defibrinated blood or after clotting, instances of its apparent avirulence being explicable by a natural resistance of the monkey; (3) that it may perhaps be possible to deprive typhus blood serum (obtained after clotting) of its virulence by prolonged centrifugation, but that this does not necessarily indicate an intraleucocytic localization of the virus; and (4) that repeated washings of the blood corpuscles do not deprive them of their infectivity, a fact explicable by the physical phenomena involved in centrifugation.

FILTERABILITY.

VIRUS IN THE BLOOD.

The question of the filterability of the virus as it exists in typhus blood has been studied by several groups of workers. Ricketts and Wilder were the first to come forward with an answer to this question. On February 5, 1910, they reported a filtration experiment. In this a monkey inoculated with filtered (Berkefeld candle) blood serum (from defibrinated blood) failed to react. Almost simultaneously Goldberger and Anderson (Feb., 18, 1910) reported a similar experiment with a like result. In a paper published some weeks later (Apr. 23, 1910) Ricketts and Wilder report apparently the same result from a second experiment.

In October, 1910, Nicolle, Conor, and Conseil reported two series of experiments with filtered serum (from clotted blood) on monkeys and a third on man. In the first, one of the two monkeys that had received the filtered serum gave no indications of a reaction; the other (bonnet 47) presented an elevation of temperature of 0.5 degree

between the sixteenth and eighteenth day,[1] so that the result was doubtful. When later subjected to an immunity test bonnet 47 proved refractory, from which they conclude that the subcutaneous inoculation of filtered typhus serum produced in this monkey a feeble thermic reaction (doubtful), which, however, conferred an absolute immunity to a subsequent immunity test.

In the second series of their experiments neither the monkey that received the unfiltered serum nor the three monkeys that received the big doses of the filtered serum presented the slightest indications of a reaction.

In a third experiment one of the authors submitted himself to a subcutaneous injection of 0.25 c. c. of filtered typhus serum without developing any symptoms.

After reviewing the results of filtration recorded by Ricketts and Wilder and by Anderson and Goldberger, Nicolle, Conor, and Conseil (January, 1911) conclude "that the serum obtained by centrifugation of defibrinated blood is always inactive after filtration; that the serum obtained after coagulation is generally also inactive after filtration, but not constantly so; and that the only hypothesis that permits of an explanation of these facts is that under ordinary conditions the unknown microbe of typhus is present in the filtrate in numbers too small to cause infection or immunization of the inoculated animal. This microbe is therefore filterable and probably intraleucocytic." It should here be noted that this was written before the result of the immunity test of the monkey used in the second of Ricketts and Wilder's filtration experiments was published. This result appeared in a paper published by Wilder in July, 1911, and showed that as in one of Nicolle, Conor, and Conseil's experiments the monkey that had received the injection of filtered (defibrinated blood) serum was resistant to the immunity test.

In September, 1911, Nicolle, Conor, and Conseil reported still another filtration experiment. Believing that the virus is intraleucocytic they thought "that it would perhaps be possible to obtain, by the artificial disintegration of a large number of these cells, enough free organisms so that their filtered emulsion would infect the monkey." This, however, did not prove to be the case and they conclude that "in this instance, again, the number of organisms that passed the filter was undoubtedly insufficient," so that their "previous positive filtration experiment remains unique." They are evidently still ignorant of Ricketts and Wilder's "positive" (?) result.

In November, 1911, Gaviño and Girard published some studies in typhus, in which they report an absolutely negative result following inoculation of a monkey with filtered (through a Berkefeld candle) peritoneal exudate rich in erythrocytes and leucocytes, particularly polynuclears. This was obtained from the peritoneal cavity of a monkey at the height of an experimental typhus three hours after injecting some peptonized broth.

Summarizing the foregoing, we have eight attempts, so far recorded, to pass the virus of typhus through a Berkefeld filter. Of these, six were negative; in one of the other two (Wilder, 1911) the monkey that had been inoculated with the filtrate, without giving any evidence of a reaction, was later found to be resistant to an immunity

[1] In their summary January, 1911, this is stated as "0.5° to 0.8° between the fifteenth and twenty-first day."

test with virulent blood; in the other (Nicolle, Conor, and Conseil, January, 1911), one of a pair of monkeys inoculated with some of the filtrate is described as having presented a doubtful reaction, and later was found resistant to an inoculation with virulent blood.

Before discussing these results and their significance, we desire to record some new experiments of our own.

Experiment No. 8.

On October 13, 1911, some blood was drawn from rhesus No. 158, then in the first day of a severe experimental typhus. After defibrination this blood was centrifugated for about half an hour, after which the supernatant serum was pipetted off. A portion of the serum was diluted with three volumes of saline solution and then passed through a Berkefeld candle, after which the following inoculations were made: Of the unfiltered serum rhesus No. 141 was given 3.5 c. c. intravenously and rhesus No. 142, 4 c. c. intraperitoneally and 0.5 c. c. subcutaneously. Of the diluted filtered serum rhesus No. 143 was given 8.5 c. c. (representing 2.12 c. c. of the original) intravenously and 6 c. c. (representing 1.5 c. c. of the original) intraperitoneally; rhesus No. 144 was given 9.5 c. c. (representing 2.4 c. c. of the original) intravenously and 5 c. c. (representing 1.25 c. c. of the original) intraperitoneally.

Result: Rhesus No. 141 (unfiltered serum), after an incubation period of 7 days, developed a sharp reaction (chart 3), on the third day of which he was sacrificed in the act of aspirating blood for passage. Rhesus No. 142 (unfiltered serum), 143 (filtrate), 144 (filtrate), during a period of observation of 29 days gave no evidence of a reaction. At the end of this period these three monkeys were subjected to an immunity test, each receiving intravenously 2.5 c. c. of defibrinated blood of rhesus No. 163, then in the second day of a marked experimental typhus. All three animals responded promptly and sharply to this test (chart 4).

It appears, therefore, that of the two monkeys (Nos. 141 and 142) that were inoculated with virulent typhus serum obtained after a half hour's centrifugation of virulent defibrinated blood (virulent for two monkeys in a dose of 3.5 c. c. each, intravenously) one (the one receiving the larger dose) failed to react without being refractory to the immunity test and that neither of the two monkeys inoculated with the filtered serum was either infected thereby or had any resistance conferred upon it.

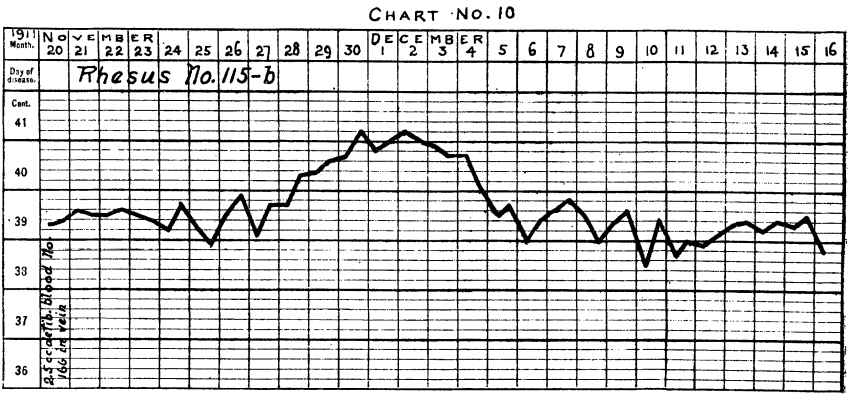
Experiment No. 9.

On October 22, 1911, blood was drawn from the carotid of rhesus No. 141, at this time in the third day of a marked attack of experimental typhus, and allowed to clot in a tube. At the end of 3 hours the tube was centrifugated for about 15 minutes in order to separate the serum more thoroughly from the clot. After drawing off the clear straw-tinted serum a portion was diluted with 3 volumes of saline solution and passed through a Berkefeld filter, after which the following inoculations were made: Of the unfiltered serum, rhesus No. 147 was given 4.5 c. c. and No. 148, 3 c. c., both intravenously. Of the dilute filtered serum rhesus Nos. 115*a* and 115*b* were each given 10 c. c. (representing 2.5 c. c. of undiluted serum) intravenously and 2 c. c. (representing 0.5 c. c. of undiluted serum) intraperitoneally.

Result: As already stated in a previous connection, rhesus No. 148 (3 c. c. serum) developed a severe typhus reaction after an incubation period of about 9 days (chart 5). Rhesus No. 147 (4.5 c. c. serum), No. 115*a* (3 c. c. filtered serum), No. 115*b* (3 c. c. filtered serum) during a period of observation of 29 days following the inoculation, presented no recognizable evidence of a reaction. At the end of this period of observation monkeys Nos. 147, 115*a* and 115*b* were subjected to an immunity test consisting of an inoculation of defibrinated blood of rhesus No. 166 then in the third day of a marked experimental typhus, No. 147 receiving 2 c. c. intravenously and 0.5 c. c. subcutaneously, No. 115*a* 2.5 c. c. subcutaneously, and No. 115*b* 2.5 c. c. intravenously. Rhesus Nos. 147 and 115*b* reacted promptly and sharply to this test (charts 6 and 10). No. 115*a*, however, gave no evidence of a reaction during a period of 33 days following this test. Sixty-two days after the inoculation with the filtered serum rhesus No. 115*a* was given a second immunity test consisting of an inoculation, part intravenous and part subcutaneous, of 2.5 c. c. of virulent defibrinated blood of

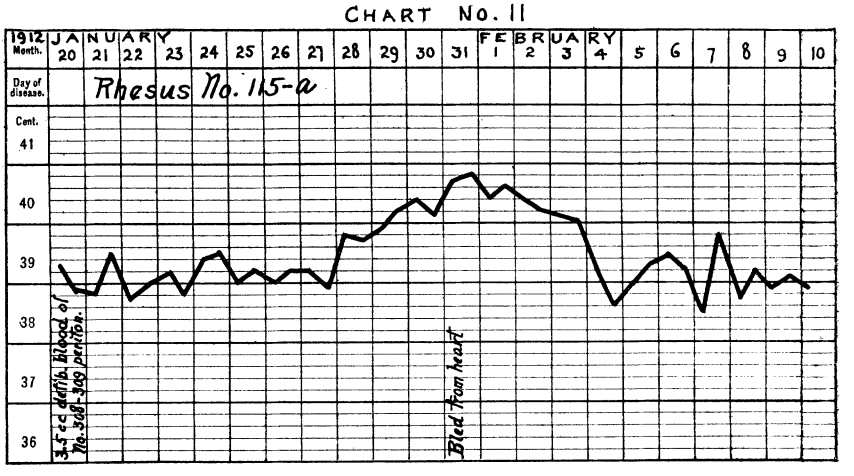
rhesus No. 176, then in the second day of a marked attack. Twenty-eight days later, or 90 days after the inoculation with filtered serum, having failed to react to the second test, this animal was subjected to a third immunity test consisting of an intravenous inoculation of 3.5 c. c. of mixed defibrinated blood from rhesus Nos. 308 and 309, both of which were then in the second day of marked experimental attacks.

Following this inoculation rhesus No. 115*a*, after an incubation period of 8 days, developed a well-marked typhus reaction (chart 11).



Temperature curve of rhesus No. 115*b*, showing reaction following immunity test.

It would appear therefore (1) that of the two animals that were inoculated with the unfiltered serum obtained after coagulation and centrifugation of virulent blood, one (the one receiving the larger dose) failed to react without, however, being resistant to the subsequent immunity test and (2) that neither of the two monkeys inoculated



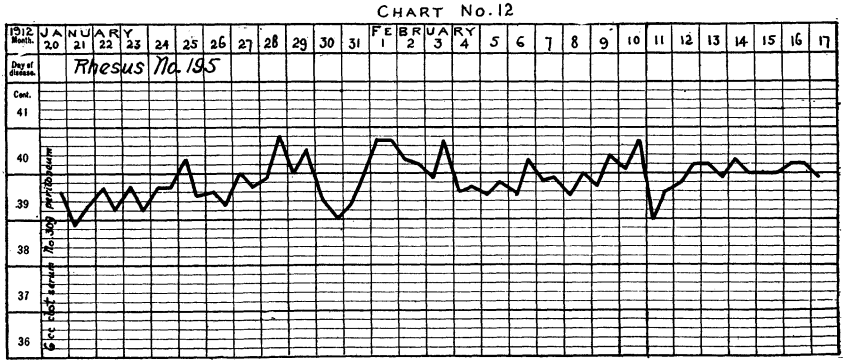
Temperature curve of rhesus No. 115*a*, showing reaction following third immunity test.

with the filtered serum was infected nor were they vaccinated by the inoculation, although the failure of one of them (115*a*) to react to two successive immunity tests might have suggested that he had been vaccinated had he not been given a third test to which he responded promptly and definitely.

Experiment No. 10.

On January 20, 1912, some blood was drawn into a tube from the carotid of rhesus No. 309, which at this time was in the second day of a sharp attack of the experimental disease. This blood was allowed to clot at room temperature. At the end of two and one half hours it was centrifugated, after which the perfectly clear serum was pipetted off and a portion diluted with 2 volumes of saline solution. This diluted serum was then passed through a Berkefeld candle, after which the following inoculations were

CHART No. 12

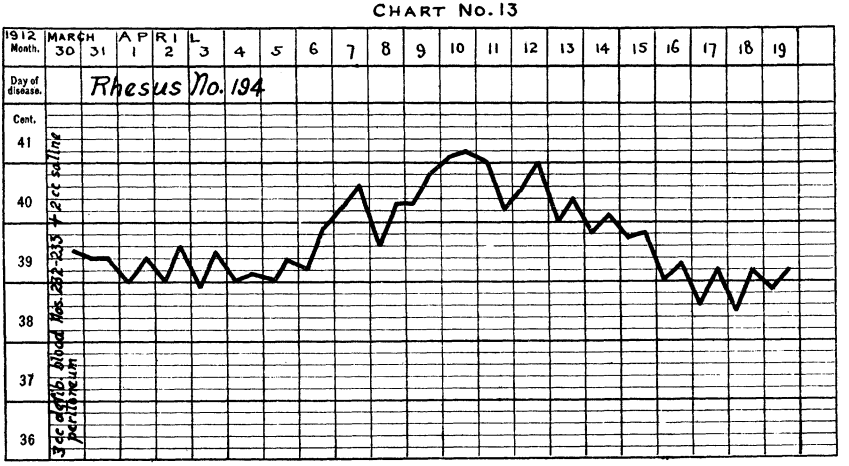


Temperature curve of rhesus No. 195, showing what was probably a reaction (complicated by tuberculosis), following an inoculation with blood serum.

made: Rhesus No. 194 was given 18 c. c. of the filtrate (representing 6 c. c. of serum) intraperitoneally; rhesus No. 195 was given 6 c. c. of unfiltered serum, likewise intraperitoneally.

Result: Rhesus No. 195 developed what was probably a typhus reaction after an incubation period of 8 days (chart 12). Some doubt, however, is cast upon this interpretation by the fact that this monkey's normal temperature was somewhat high, due, probably, to a coexisting tubercular infection that later was found to be present.

CHART No. 13

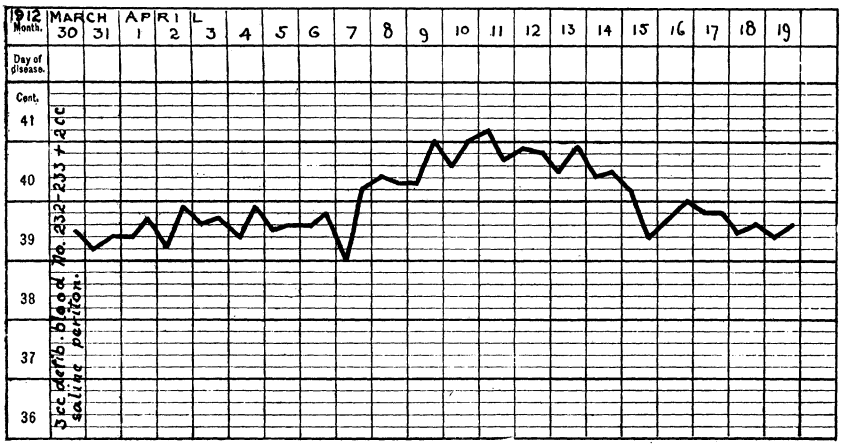


Temperature curve of rhesus No. 194, showing reaction following second immunity test.

Rhesus No. 194 presented no indications of a reaction during a period of observation of 34 days. At the end of this time he was subjected to an immunity test consisting of an intraperitoneal injection of 6 c. c. of virulent defibrinated blood without eliciting any evidence of a reaction. This would suggest that this monkey had been made refractory by the inoculation with the filtrate, just as appeared to have happened in one instance in Rickets and Wilder's experience (Wilder, 1911) and in one instance in

the experience of Nicolle, Conor, and Conseil (January, 1911). On subjecting this monkey to another immunity test (3 c. c. defibrinated blood intraperitoneally), however, 70 days after the inoculation with the filtered serum rhesus No. 194 responded promptly and sharply as may be seen from chart 13.

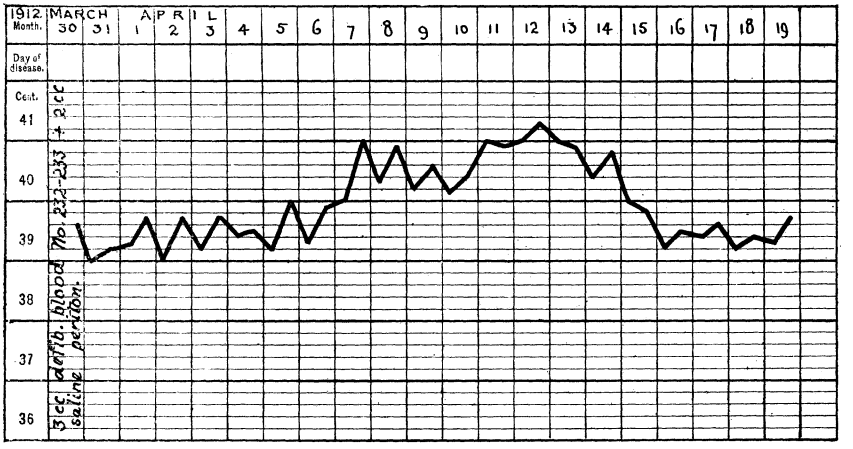
CHART No. 14



Temperature curve of rhesus No. 223, showing reaction following immunity test.

It would appear therefore that the inoculation of rhesus No. 194 with 6 c. c. of filtered serum obtained after centrifugation of clotted typhus blood had neither infected nor vaccinated this animal.

CHART No. 15



Temperature curve of rhesus No. 224, showing reaction following immunity test.

Experiment No. 11.

On March 6, 1912, blood was aspirated from rhesus No. 210 and No. 213. The former was in the seventh and the latter in the fifth day of an experimental typhus. The blood was allowed to clot. After 3 hours it was centrifugated in order better to separate the serum from the clot. The serum was then pipetted off and the two specimens were mixed. A portion of this serum was diluted with 3 volumes of saline

solution and then passed through a Berkefeld filter, after which the following inoculations were made:

Rhesus No. 221 received 6 c. c. of unfiltered serum, rhesus Nos. 223 and 224 each received 6 c. c. of filtered serum intraperitoneally.

Result: None of these animals presented any evidence of a reaction following this inoculation. Twenty-four days later the three were subjected to an immunity test consisting of an intraperitoneal injection of 3 c. c. of virulent defibrinated blood of rhesus Nos. 232 and 233, diluted with 2 c. c. of saline solution. Following this rhesus Nos. 223 and 224 responded promptly and sharply (charts 14 and 15), but No. 221 gave no evidence of a reaction. Rhesus No. 221 has been subjected to two more immunity tests since, but so far has presented no evidence of a reaction. (Cf. Exp. 5.)

It would appear, therefore, that the inoculation of two monkeys with filtered serum obtained after centrifugation of clotted typhus blood had produced neither infection nor immunization in either.

Summarizing our attempts to infect the rhesus monkey with filtered typhus blood serum, we find that in no instance was infection produced, and that when subjected to an immunity test 2 (rhesus 115a, experiment 2, and rhesus 194, experiment 3) of the 7 monkeys employed at first appeared to be resistant (apparently vaccinated), but later when the test was repeated both responded.

DISCUSSION.—Having presented first a summary of the results of the filtration experiments recorded in the literature and then detailed our own attempts to pass the virus of typhus through the Berkefeld filter, we may proceed with a consideration of their interpretation and significance.

The significance to be attached to the results of the filtration experiments, as a whole, evidently hinges on the interpretation to be given to the observation that some of the monkeys that had been inoculated with filtered typhus blood serum were subsequently resistant to an immunity test, although the inoculation with the filtrate had not been followed by any definite evidence of a febrile reaction. Nicolle, Conor, and Conseil (October, 1910, p. 3) interpret the resistance to the immunity test of one of their monkeys as indicating an immunization caused by "the microbe of typhus, which is very small and passes the Berkefeld filter"; whereas, Wilder suggests that a similar result in Ricketts's and Wilder's experience "may be explained by one or more of several different hypotheses. The animal may have been naturally immune to typhus. If such is the case, however, he is the first normal monkey with which we have had to deal that has shown such an absolute immunity when inoculated with over 1 c. c. of virulent blood. A second possibility is that the animal was immunized by the filtered serum." "Such immunization could have been accomplished either by microorganisms sufficiently small to pass the filter, by fragments of organisms, or by toxins." Although Wilder is evidently disinclined to attach any importance to the idea that the normal monkey may be naturally immune to typhus, and Nicolle, Conor, and Conseil do not even consider it, the results of our inoculations with virulent typhus blood serum (unfiltered), as well as the final results of the repeated immunity tests, clearly show that something in the nature of a natural immunity or transient unresponsiveness to typhus is possessed by some normal monkeys and we believe this to be the true explanation of the instance noted by Nicolle, Conor, and Conseil and that by Ricketts and Wilder. We believe that, had they repeated the immunity tests in these monkeys as we did in ours, their results would probably have shown

as did ours that these animals were only apparently (? transiently) resistant. These monkeys were therefore not immunized by the filtered typhus serum.

CONCLUSIONS.—It is permissible to conclude from the foregoing that there is no evidence to show that the virus in the blood of typhus is able to pass the Berkefeld filter; and, incidentally, that virulent typhus blood contains no toxin, or contains it in quantities too small to cause an appreciable increase in the normal resistance of the monkey when injected, even repeatedly, in the ordinary doses.

THE VIRUS IN THE LOUSE.

The fact that the organism of typhus, as it occurs in the blood, does not appear capable of passing the Berkefeld filter does not of necessity mean that it may not be capable of passing in the form in which it exists in the body of the louse. It was decided, therefore, to put this idea to the test of experiment.

Experiment No. 12.

On December 3, 1911, 82 living body lice of group No. 7—M and 83 of group No. 8—M were crushed and rubbed up in 16.5 c. c. of saline solution. Of this suspension 11 c. c. were diluted with an equal volume of saline solution and filtered through a Berkefeld filter. With 15 c. c. of the clear filtrate, representing 75 body lice, rhesus No. 311 was inoculated by an injection of about 13 c. c. intraperitoneally and about 2 c. c. subcutaneously. Control inoculations with the original (undiluted) suspension (reported in a previous communication by Goldberger and Anderson, Mar. 1, 1912) were made in two rhesus monkeys; one, No. 308, received 3.5 c. c. (representing 35 lice), and No. 309, 1.5 c. c. (representing 15 lice) subcutaneously.

Body lice of group No. 7—M had been allowed to feed daily during the 6 days immediately prior to the date of the experiment on various cases of typhus fever in the Hospital General, Mexico City. They were last applied to case No. 16—M, in the eleventh day of illness, at 10.50 to 11.20 a. m. December 2. Body lice of group No. 8—M were insects that had been allowed to feed daily during the 5 days immediately preceding the date of the experiment on various cases of typhus fever. They were last applied to case No. 16—M, in the eleventh day of illness, at 10.20 to 10.50 a. m., December 2. Throughout the period during which they were allowed to feed on patients with typhus the lice of groups Nos. 7—M and 8—M were kept at room temperature (about 14 to 24° C.).

Result: As has already been reported, the controls—rhesus Nos. 308 and 309—presented no evidence of a typhus reaction. No. 311 developed a markedly remittent temperature following the inoculation with the filtrate, and later was found to be infected with tuberculosis. This experiment has, therefore, no significance, so far as filtration is concerned.

Experiment No. 13.

The preceding experiment was repeated on December 10. At 5.30 p. m. 478 lice of group No. 9—M were crushed and rubbed up in 48 c. c. of saline solution. Of this suspension about 7.5 c. c. (representing about 73 lice) were subsequently injected in rhesus No. 320. Another portion of the suspension, after dilution with 2 volumes of saline solution, was filtered through a Berkefeld filter, and then 39 c. c. of the filtrate, representing not less than 130 lice, were injected intraperitoneally in rhesus No. 319.

The lice composing group No. 9—M were body lice that had been applied daily and been allowed to feed on various cases of typhus at the Hospital General, Mexico City, during the 6 days immediately prior to the date of the experiment. Throughout this period they were kept at room temperature (14° to 24° C.). They were last applied on December 9 between 10.15 a. m. and 12 m. to case No. 19—M, in the seventh day of illness.

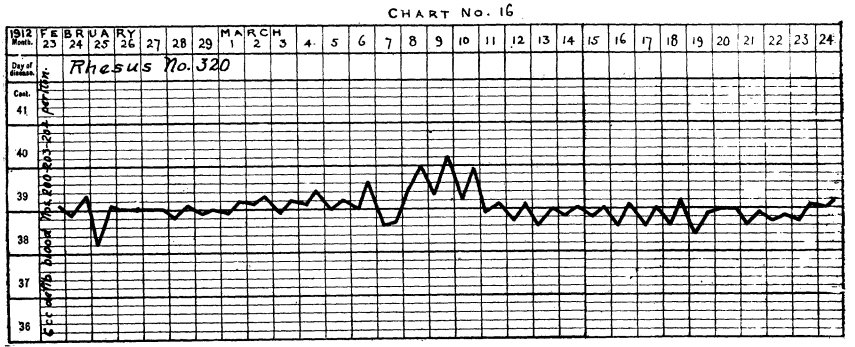
Result: As already reported (Goldberger and Anderson, 1912), rhesus No. 320 presented no evidence of a typhus reaction following the inoculation with the crushed lice suspension. This animal, after resisting two successive immunity tests with defibrinated blood, was subjected to a third test February 23, 1912, or 75 days after inoculation with the lice suspension. This consisted of an intraperitoneal inocula-

tion of 6 c. c. of virulent defibrinated blood. During 3 days, beginning 14 days after this inoculation, rhesus No. 320 presented a well-defined perturbation in temperature that may have represented an abortive typhus reaction (chart 16).

Rhesus No. 319 presented no evidence of a reaction following the inoculation with the filtrate. Thirty-one days later he was subjected to an immunity test consisting of an intravenous inoculation of 3 c. c. of virulent defibrinated blood of rhesus No. 187; 21 days after this, having in the meantime presented no evidence of a typhus reaction, he was subjected to a second test consisting of an intravenous injection of 2.5 c. c. of virulent defibrinated blood of rhesus No. 115*a*. Not presenting any evidence of a reaction, he was subjected to a third test on February 23, 75 days after the inoculation with the filtered lice suspension. This test, as in the case of rhesus No. 320, consisted of an intraperitoneal injection of 6 c. c. of virulent defibrinated blood. During a period of observation of 33 days following this, rhesus No. 319 presented no recognizable indications of a typhus reaction.

DISCUSSION.—The immunity tests given monkeys No. 319 and No. 320 would indicate the possession of a complete insusceptibility to typhus by the former and an almost if not quite complete insusceptibility by the latter. In the early stages of our work we would have been inclined to interpret this resistance as due to an immunization induced by the original inoculation of filtered and unfiltered

CHART No. 16



Temperature curve of rhesus No. 320, showing abortive (?) reaction following third immunity test.

lice suspensions, respectively. Our more recent experience, however, having developed evidence that a healthy monkey may from time to time be encountered that is naturally resistant, a definite interpretation of the result of this single experiment is not permissible. Nevertheless, we undoubtedly have here a suggestion of a filterable phase in the body of the louse.

With the idea of obtaining a clearer conception of the nature of the virus of typhus we have studied its resistance to drying and to certain extremes of temperature.

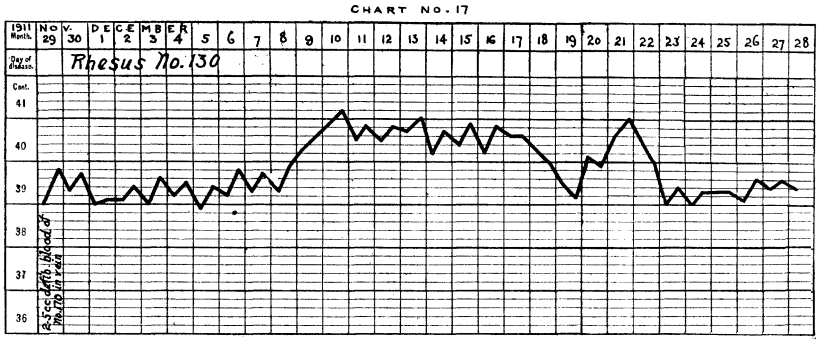
DRYING.

Experiment No. 14.

At 12 m., October 22, 1911, 3 c. c. of virulent defibrinated blood of rhesus No. 141 (then in the third day of an experimental typhus) were put in one petri dish and 6 c. c. in another. The dishes with the lids tilted were placed over sulphuric acid in a desiccator from which the air was then exhausted. This apparatus with the blood was then placed at a temperature of from about 15° to 19° C. At the end of 25 hours the vacuum was broken and the dish containing the 3 c. c. of blood was removed, after which the air in the apparatus in which the dish with the 6 c. c. of blood remained was again exhausted and the apparatus continued as before.

The 3 c. c. of blood were found to have been dried and to have formed a scale on the bottom of the dish. The dry blood was rubbed up in saline solution and injected intraperitoneally into rhesus No. 130.

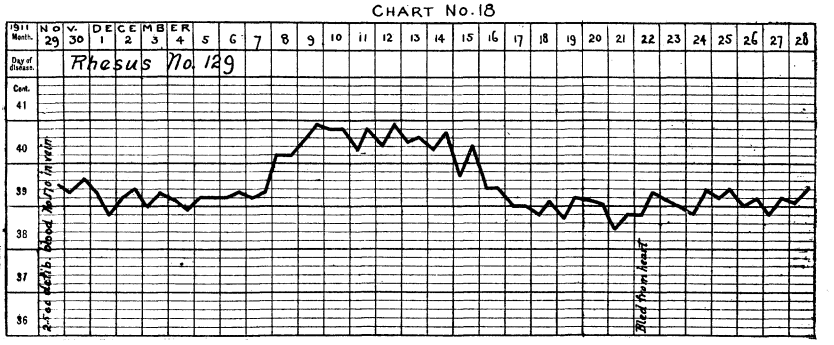
At the end of 6 days, that is, on October 28, the dish containing the 6 c. c. of blood was removed from the desiccator. The blood was found dried into a scale. This dried blood was rubbed up in saline solution and injected intraperitoneally into rhesus No. 129.



Temperature curve of rhesus No. 130, showing reaction following immunity test.

Result: Neither rhesus No. 130 nor No. 129 presented any evidence of a reaction. Thirty-eight days after the inoculation of the former and 32 of the latter, both were subjected to an immunity test consisting of an intravenous injection of 2.5 c. c. of defibrinated blood of rhesus No. 170, then in the fourth day of an attack of typhus. Both monkeys promptly responded to this test, No. 130 developing a grave reaction, and No. 129 a reaction of moderate severity (charts 17 and 18).

Monkeys Nos. 130 and 129 were therefore neither infected nor immunized by the inoculation, in the one of 3 c. c. of virulent defibrinated blood after drying 25 hours, and in the other of 6 c. c. after drying 6 days.



Temperature curve of rhesus No. 129, showing reaction following immunity test.

Experiment No. 15.

At 2 p. m., February 12, 1912, 3 c. c. of mixed virulent blood of rhesus Nos. 196, 198, and 322 was put in a petri dish, and the dish, with the cover tilted, placed over sulphuric acid in a desiccator as in experiment No. 14. After exhausting the air the apparatus was placed at 15° C. At 2 p. m., February 13—that is, after 25 hours—the dish with the blood now dried to a scale was removed from the apparatus. The blood was then rubbed up in saline solution and injected intraperitoneally into rhesus No. 208.

Result: During a period of observation of 39 days monkey No. 208 presented no evidence of a reaction. Forty-six days after the inoculation this monkey was subjected to an immunity test consisting of an intraperitoneal injection of 3 c. c. of defibrinated blood of rhesus No. 234 diluted with 2 c. c. of saline solution. To this test monkey No. 208 reacted promptly and sharply.

It would appear, therefore, that the inoculation with 3 c. c. of virulent defibrinated blood after drying for 25 hours had neither infected nor immunized this animal.

CONCLUSIONS.—The negative outcome of these two experiments would indicate that the typhus virus can not survive drying, or, more accurately, that it loses its virulence when dried for 25 hours under the conditions described. Unfortunately the nature of the problem does not permit final conclusions to be drawn from so small a number of experiments.

HEAT.

Gaviño and Girard were the first to study the resistance of the typhus virus to heat. In May, 1910, they inoculated a monkey with 10 c. c. of defibrinated typhus blood after heating at 50° for 40 minutes. The monkey so inoculated developed a well-marked febrile reaction after an incubation period of 14 days.

In a second experiment, performed three months later, they inoculated a monkey with 4 c. c. of defibrinated blood after heating at 55° for 15 minutes. This inoculation not being followed by any evidence of a reaction, Gaviño and Girard concluded that the virulence of the blood was destroyed by the heating to which they had subjected it. The immunity of this animal was not tested until later; when tested 56 days after the inoculation of the heated blood with 3 c. c. of defibrinated typhus blood the monkey gave no evidence of a reaction. Commenting on this result, Gaviño and Girard state that it would seem as if the first noninfecting injection with heated blood vaccinated against a subsequent injection with virulent blood, although they add that the objection might be raised that they had encountered in this animal one that was naturally immune.

In October, 1910, Nicolle, Conor, and Conseil reported an experiment in which the intraperitoneal injection of 4 c. c. of citrated blood (virulent for the controls) after heating at 50° C. for 15 minutes conferred no resistance to a subsequent immunity test. They concluded from this that the virus is destroyed by heating at 50° for 15 minutes. In view of the positive result obtained by Gaviño and Girard, and in the light of the fact several times referred to in other portions of this paper that the monkey is not invariably responsive to an infective inoculation, this conclusion is not permissible.

In a paper published in November, 1911, Gaviño and Girard report additional tests of the resistance of the typhus virus to heat. In one experiment they inoculated three guinea pigs intraperitoneally, each with 3 c. c. of defibrinated typhus blood heated at 55° for 15 minutes. None of these pigs presented any indications of a reaction. The immunity test, if made, is not recorded. In a second experiment they inoculated each of two monkeys with 7 c. c. of defibrinated typhus blood after heating at 55° for 15 minutes. Not presenting any evidence of a reaction, they were subjected to an immunity test one month later. To this both monkeys gave marked reactions. Consequently they conclude that typhus blood heated for 15 minutes at 55° loses its virulence and that a noninfecting injection of heated

blood does not vaccinate against a subsequent inoculation with virulent blood.

The heating experiments recorded in the literature may be summarized as follows:

At 50° for 40 minutes.—One experiment, 1 monkey; blood retained its virulence (Gaviño and Girard).

At 50° for 15 minutes.—One experiment, 1 monkey; not infected, not immunized (Nicolle, Conor, and Conseil).

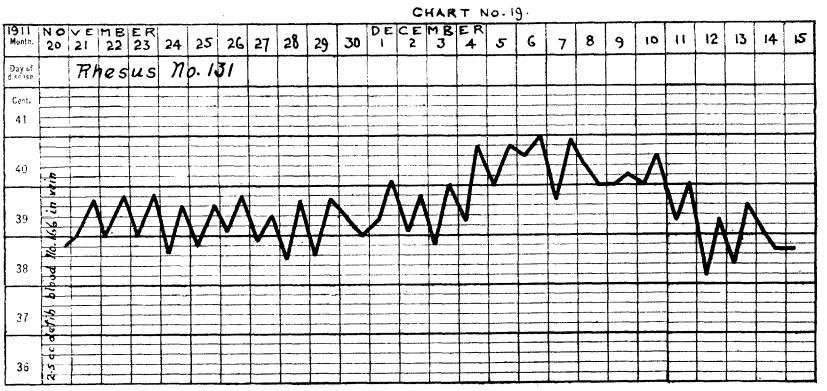
At 55° for 15 minutes.—One experiment, 1 monkey; not infected, but resisted immunity test (Gaviño and Girard). One experiment, 3 guinea pigs; not infected; immunity test not recorded (Gaviño and Girard). One experiment, 2 monkeys; not infected, not immunized (Gaviño and Girard).

Before attempting to interpret these results, we desire to record some experiments of our own.

Experiment No. 16.

On October 22, 1911, we heated some virulent defibrinated blood of rhesus No. 141 in a tube at 55° for 15 minutes and with it inoculated 2 monkeys: Rhesus No. 131, received 3.5 c., c., and rhesus No. 132, 3 c., c., both intravenously.

Result: Neither of this pair of monkeys presented any evidence of a reaction following their inoculation, although 3 other monkeys, inoculated at the same time



Temperature curve of rhesus No. 131, showing reaction following immunity test.

with like doses of the unheated blood, all developed well marked typhus reactions. Twenty-nine days later rhesus Nos. 131 and 132 were subjected to an immunity test consisting in each of an intravenous injection of 2.5 c. c. of defibrinated blood of rhesus No. 166, then in the third day of a typhus reaction. Both animals reacted sharply (charts 19 and 20); rhesus No. 131, after an incubation of 14 days with a fever of 8 days' duration, and rhesus No. 132 after an incubation of 8 days with a fever of 6 days' duration.

The two animals were therefore neither infected nor immunized.

Experiment No. 17.

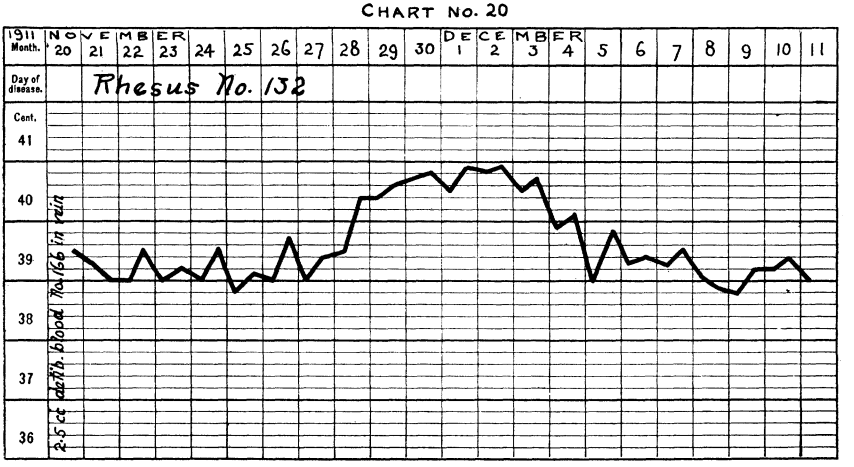
On February 14, 1912, we heated some virulent defibrinated blood in a tube at 60° for 5 minutes and injected 3 c. c. of it into the peritoneal cavity of rhesus No. 199.

Result: During a period of observation of 23 days monkey No. 199 gave no indications of a reaction. Tested at the end of this period by an intraperitoneal injection of 6 c. c. of virulent defibrinated blood, rhesus No. 199 developed a well marked febrile reaction.

This animal was therefore neither infected nor immunized by the injection of 3 c. c. of blood heated at 60° for 5 minutes.

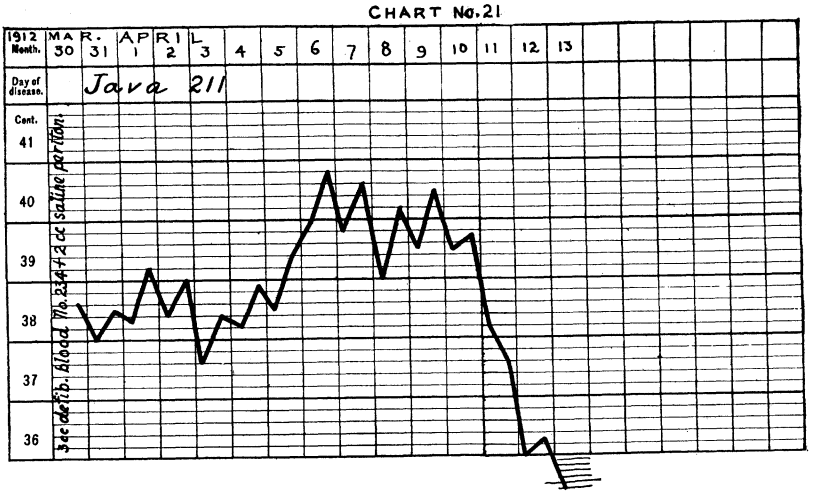
Experiment No. 18.

On February 23 we gave Java monkey No. 211 two intraperitoneal injections of heated defibrinated typhus blood; the first of 6 c. c. heated by mistake at 60° for 5 minutes, and the second also of 6 c. c. heated at 55° for 5 minutes.



Temperature curve of rhesus No. 132, showing reaction following immunity test.

Result: Following these injections monkey No. 211 manifested no evidence of a reaction. An immunity test given 36 days later consisting of an intraperitoneal injection of 3 c. c. of defibrinated blood of rhesus No. 234, then in the second day of an experimental typhus, diluted with 2 c. c. of saline solution, was followed after an incubation period of 6 days by a typhus reaction terminating in death on the ninth day (chart 21).



Temperature curve of Java No. 211, following immunity test.

It would appear therefore that this monkey was neither infected nor immunized by the intraperitoneal injection of 6 c. c. of blood heated at 55° for 5 minutes nor by that at 60° for 5 minutes.

Experiment No. 19.

On March 6, 1912, we heated some virulent blood in a tube at 55° for 5 minutes and injected 6 c. c. of it into the peritoneal cavity of rhesus No. 220.

Result: During a period of observation of 24 days this animal presented no recognizable indications of a reaction. Tested at the end of this time by an intraperitoneal injection of 3 c. c. of defibrinated blood of rhesus No. 234, diluted with 2 c. c. of saline solution, monkey No. 220 promptly developed a severe reaction (chart 22).

It would appear, therefore, that monkey No. 220 was neither infected nor immunized by the intraperitoneal injection of 6 c. c. of typhus blood heated at 55° for 5 minutes.

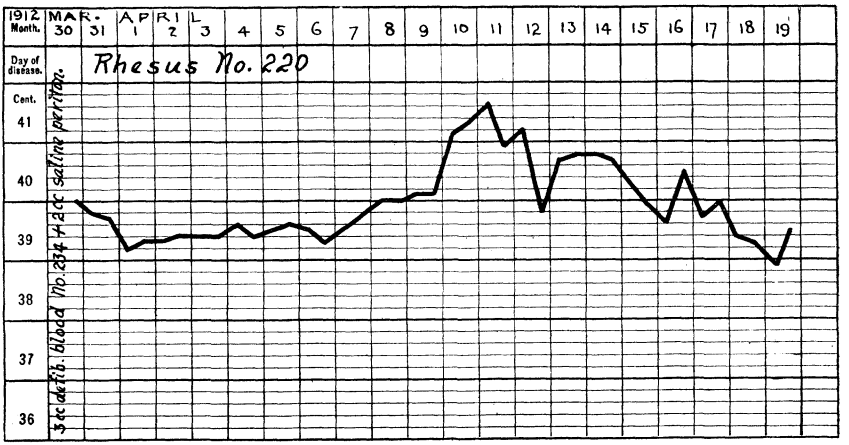
Our experiments may be summarized as follows:

At 55° for 15 minutes.—One experiment; neither infection nor immunization in 2 monkeys.

At 60° for 5 minutes.—One experiment; 1 monkey; neither infection nor immunization.

At 60° for 5 minutes and 55° for 5 minutes, combined.—One experiment; 1 monkey; neither infection nor immunization.

CHART No. 22



Temperature curve of rhesus No. 220, showing reaction following immunity test.

At 55° for 5 minutes.—One experiment; 1 monkey; neither infection nor immunization.

Combining the summary of the experiments previously recorded with that of our own, we have the following:

50° for 40 minutes.—One experiment, 1 monkey; blood retained its virulence (Gaviño and Girard).

50° for 15 minutes.—One experiment, 1 monkey; not infected, not immunized (Nicolle, Conor, and Conseil).

55° for 15 minutes.—One experiment, 1 monkey; not infected, but resisted immunity test (Gaviño and Girard).

One experiment, 3 guinea pigs; not infected; immunity test not reported (Gaviño and Girard).

One experiment, 2 monkeys; not infected, not immunized (Gaviño and Girard).

One experiment, 2 monkeys; neither infection nor immunization (Goldberger and Anderson).

55° for 5 minutes.—One experiment, 1 monkey; neither infection nor immunization (Goldberger and Anderson).

55° for 5 minutes combined with 60° for 5 minutes.—One experiment, 1 monkey; neither infection nor immunization (Goldberger and Anderson).

60° for 5 minutes.—One experiment, 1 monkey; neither infection nor immunization (Goldberger and Anderson).

Reviewing this summary, we find one experiment by Nicolle, Conor, and Conseil, in which blood heated at 50° for 15 minutes neither infected nor immunized, and one by Gaviño and Girard in which blood heated at 55° for 15 minutes did not infect but apparently immunized, which appear to be out of harmony with the other results. If we bear in mind the possibility, to which we have elsewhere in this paper called attention, that the normal monkey may at times fail to respond to the first and sometimes to the second inoculation with virulent material, we will have (what we believe is) the explanation of this lack of harmony.

CONCLUSIONS.—The determination of the resistance of typhus virus to heat is obviously beset with the same difficulties as the determination of its resistance to drying. Our inability to cultivate the organism hardly permits of the final determination of these points. Nevertheless, when we combine the results of our own heating experiments with those previously recorded we have a body of facts that justify certain conclusions that may be summarized as follows:

1. The virus may (perhaps generally does) retain its virulence after heating at 50° for 40 minutes.
2. The virus is deprived of virulence (? killed) by heating at 55° for 15 minutes.
3. The virus is probably deprived of virulence (? killed) by heating at 60° for 5 minutes.
4. The virus is probably deprived of virulence (? killed) by heating at 55° for 5 minutes.

FREEZING.

So far as we are aware, the following attempts to determine the resistance of the typhus virus to "freezing" are the first to be recorded:

Experiment No. 20.

At 12.30 p. m., October 22, 1911, some defibrinated blood of rhesus No. 141 (chart 3), then in the third day of a typhus reaction, was put into a freezing mixture of ice and hydrochloric acid and frozen solid. At 2.30 p. m. it was removed from this mixture and placed in a tank of brine at 0° C. At 1.30 p. m., October 23, it was taken out of the brine, rapidly thawed and, after warming slightly, 3 c. c. of it were intraperitoneally injected into rhesus No. 155.

Result: After an incubation period of 8 days this monkey developed a well-marked typhus reaction.

The infectivity of the blood was, therefore, not destroyed by "freezing" for 25 hours.

Experiment No. 21.

On February 12, 1912, some virulent defibrinated blood from rhesus Nos. 196, 198, and 322 was frozen as in the preceding experiment and put in a tank of brine at 0° C. The blood was allowed to remain at this temperature for 8 days—that is, till February 20; it was then removed and allowed to thaw at room temperature. Having thawed, it was warmed slightly, and 6 c. c. of it was injected intraperitoneally into rhesus No. 210.

Result: After an incubation period of 9 days, rhesus No. 210 developed a well-defined typhus reaction of about 8 days' duration. He later resisted an immunity test consisting of an intraperitoneal injection of 3 c. c. of defibrinated blood of rhesus No. 324, diluted with 2 c. c. of saline solution.

It appears, therefore, that the infectivity of the blood was not destroyed by "freezing" for 8 days.

Experiment No. 22.

On February 23, 1912, some virulent blood obtained from rhesus Nos. 200, 203, and 204 was frozen and placed in brine at 0° C., as in the preceding experiments. It was kept in the brine at 0° C. for 15 days until March 9, when it was removed and permitted to thaw at room temperature. After warming slightly, 6 c. c. of it was injected intraperitoneally into Java monkey, No. 226.

Result: During a period of observation of 30 days this animal presented no evidence of a reaction. Thirty-two days after the inoculation monkey No. 226 was subjected to an immunity test consisting of an intraperitoneal injection of 3 c. c. of defibrinated blood of rhesus No. 216, diluted with 2 c. c. of saline solution. To this it responded promptly with a severe typhus reaction.

Virulent blood, after "freezing" for 15 days, did not therefore infect or vaccinate.

CONCLUSIONS.—The foregoing experiments are not extensive enough to define more than roughly the degree of resistance of the typhus virus to freezing. It is clear, however, that typhus blood may retain its infectivity after freezing (0° C.) for at least 8 days.

SUMMARY AND CONCLUSIONS.

1. The literature bearing on the duration of the infectivity of the blood is critically reviewed. Two experiments are reported, and the following conclusions are drawn:

(*a*) The blood of the monkey infected with typhus may be virulent in the prefebrile stage, but no satisfactory evidence of that fact has as yet been adduced.

(*b*) The blood of the monkey may still be virulent 24 to 32 hours after the return of the temperature to normal.

2. The literature bearing on the question of the localization of the virus is critically reviewed, and it is pointed out that the evidence adduced by Nicolle, Conor, and Conseil, in support of their hypothesis of an intraleucocytic localization, is not valid. New experiments are detailed, and the following conclusions are drawn:

(*a*) The available evidence favors the view that the typhus virus is extracellular and free in the circulating plasma.

(*b*) The serum of virulent typhus blood is constantly infective whether obtained from defibrinated blood or after clotting, instances of its apparent avirulence being explicable by a natural resistance of the monkey.

(*c*) It may perhaps be possible to deprive typhus blood serum of its virulence by prolonged centrifugation; but this does not necessarily indicate an intraleucocytic localization of the virus.

(*d*) Repeated (three) washings of the blood corpuscles does not deprive them of their infectivity.

3. The literature bearing on the question of the filterability of the typhus virus is critically reviewed. It is found that eight attempts have been recorded to pass the virus through the Berkefeld filter. Of these, six were clearly negative; in one of the other two, the monkey,

without giving any evidence of a reaction to the inoculation, was later found to be resistant to an immunity test; in the other, one of a pair of monkeys is described as having presented a doubtful reaction to the inoculation and later was found resistant to a single immunity test.

New filtration experiments are reported, but in no instance was infection produced; when submitted to an immunity test, two of seven monkeys at first appeared to be resistant, but later, when the test was repeated, both responded.

The conclusion is drawn that there is no evidence to show that the virus in the blood of typhus is able to pass the Berkefeld filter; and, incidentally, that virulent typhus blood contains no toxin, or that it contains it in quantities too small to cause an appreciable increase in the normal resistance of the monkey when injected, even repeatedly, in the doses ordinarily employed for inoculating the monkey.

Two attempts are recorded to filter the virus as it exists in the body of the louse; in one, the monkey inoculated with the filtrate, without giving any indications of a reaction, was subsequently found refractory to repeated immunity tests, suggesting that he had been vaccinated by the filtrate. While this seems to point to the existence of a filterable phase in the body of the louse, it can not be regarded as conclusive without further corroborative work.

4. The resistance of the virus to drying is tested in two experiments with results indicating that the virus is deprived of virulence at the end of 25 hours.

5. The literature bearing on the resistance of the virus to heat is critically reviewed. The results recorded are summarized, some new experiments detailed, and the following conclusions drawn:

(*a*) The virus may (perhaps generally does) retain its virulence after heating at 50° for 40 minutes.

(*b*) The virus is deprived of virulence (? killed) by heating at 55° for 15 minutes.

(*c*) The virus is probably deprived of virulence (? killed) by heating at 60° for 5 minutes.

(*d*) The virus is probably deprived of virulence (? killed) by heating at 55° for 5 minutes.

6. The resistance of the virus to freezing (0° C.) is tested. It is found that it may retain its infectivity, after freezing (0° C.), for at least 8 days.

REFERENCES.

Anderson, John F., and Goldberger, Joseph. On the relation of Rocky Mountain spotted fever to the typhus fever of Mexico. A preliminary note. Public Health Reports, xxiv, Dec. 10, 1909, p. 1861.

Same. A note on the etiology of "tabardillo," the typhus fever of Mexico. Public Health Reports, xxiv, Dec. 24, 1909, p. 1941.

Same. On the infectivity of tabardillo or Mexican typhus for monkeys and studies on its mode of transmission. Public Health Reports, xxv, Feb. 18, 1910, p. 177.

Same. On the etiology of tabardillo or Mexican typhus. An experimental investigation. Jour. Med. Research, Boston, June, 1910, p. 469–481.

Gaviño, A., and Girard, J. El tifo experimental en los monos inferiores, nota preliminar; el tifo exantemático en los monos inferiores, segunda nota. Publicaciones del Instituto Bacteriológico Nacional, México, 1910.

Same. Tercera nota sobre el tifo experimental en los monos inferiores. Publicaciones del Instituto Bacteriológico Nacional, México, Aug. 23, 1910.

Same. Cuarta nota sobre el tifo experimental en los monos inferiores. Ibid., Nov. 9, 1910.

Same. Estudio experimental sobre el tifo exantemático. Ibid., Nov. 12, 1911.

GOLDBERGER, JOSEPH, and ANDERSON, JOHN F. The transmission of typhus fever, with especial reference to transmission by the head louse (Pediculus capitis). Public Health Reports, xxvii, Mar. 1, 1912.
MOCZUTKOWSKI, O. O. Ueber die Impfbarkeit des Typhus exanthematicus. Allg. med. Cent.-Zeit., Berlin, 1900, p. 1055–1057.
NICOLLE, CH. Reproduction expérimentale du typhus exanthématique chez le singe. C. R. Acad. Sci., Paris, July 12, 1909, p. 157–160.
Same. Recherches expérimentales sur le typhus exanthématique entreprises à l'Institut Pasteur de Tunis pendant l'année 1909. Ann. de l'Inst. Pasteur, Paris, Apr., 1910, p. 243–275.
NICOLLE, CH., and CONSEIL, E. Reproduction expérimentale du typhus exanthématique chez le macaque par inoculation directe du virus humain. C. R. Acad. Sci., Paris, July 18, 1910, p. 258–260.
Same. Données expérimentales nouvelles sur le typhus exanthématique. C. R. Acad. Sci., Paris, Aug. 8, 1910.
NICOLLE, CH., CONOR, A., and CONSEIL, E. Sur quelques propriétés du virus exanthématique. C. R. Acad. Sci., Paris, Oct. 17, 1910, p. 685–688.
NICOLLE, CH., CONSEIL, E., CONOR, A., and JAEGGY, E. Recherches sur le typhus exanthématique entreprises à l'Institut Pasteur, Tunis, pendant l'année 1910. Mémoire d'ensemble. Ann. de l'Institut Pasteur, Paris, Jan., 1911, p. 1–103.
NICOLLE, CH., CONSEIL, E., and CONOR, A. Le typhus expérimental du cobaye. C. R. Acad. Sci., Paris, June 6, 1911.
NICOLLE, CH., CONOR, A., and CONSEIL, E. Sur la nature et la siege de l'agent pathogène du typhus exanthméatique. C. R. Acad. Sci., Paris, Sept. 18, 1911.
Same. Recherches Experimentales sur le typhus exanthématique entreprises a l'institut Pasteur de Tunis pendant l'année 1911. Ann. de l'Institut Pasteur Paris Apr. 25, 1912.
OTERO, M. In "Documentos relativos al concurso abierto para estudiar la etiologia y seroterapia de tabardillo." Dictamen de la Comision. Gaceta Medica de Mexico, Appendice, 1908.
RICKETTS, HOWARD T., and WILDER, RUSSELL M.: The typhus fever of Mexico (tabardillo). Preliminary observations. Jour. Am. Med. Assn., Chicago, Feb. 5, 1910, p. 463–467.
Same. The etiology of the typhus fever (tabardillo) of Mexico City. A further preliminary report. Journ. Am. Med. Assn., Apr. 23, 1910, p. 1373–1375.
WILDER, RUSSELL M.: The problem of transmission in typhus fever. Journ. infec. diseases, Chicago, July, 1911, p. 9–101.

SANITARY ADVICE FOR KEEPERS OF SUMMER RESORTS.

By W. C. Rucker, Assistant Surgeon General, Public Health and Marine-Hospital Service.

Until comparatively recent years if a keeper of a summer resort provided good housing and feeding facilities, suitable and proper social diversions, and a reasonable amount of rest and quiet, the keeper of the resort considered that he had done his duty well, and other things being equal was reasonably sure that he would have a fair return for his investment and effort. While all of these points are of no mean weight in the minds of persons who go to the seashore, the mountains, or the country to avoid the heat and discomforts of the city, they have become secondary, and the question which is now asked first of all is as to the healthfulness of the resort in question. Aside from the financial loss which the manager of a summer pleasure ground may sustain from an outbreak of sickness among his guests, there is a moral obligation to protect and further as far as possible their health interests.

In a general way it may be stated that disease is carried from the sick to the well, either directly by persons or indirectly by food and drink, dust, and insects. If the avenues for disease germs are closed